

SANDIA NATIONAL LABORATORIES
CHEMICAL & DISPOSAL ROOM PROCESSES DEPARTMENT 6748
WASTE ISOLATION PILOT PLANT PROJECT

TOP 539

CALIBRATION, USE, AND MAINTENANCE OF
THE N4MD SUB-MICRON PARTICLE ANALYZER

Revision 0

Effective Date: 1/5/96

Author:	<u>John W. Kelly</u>	<u>John W. Kelly</u>	<u>12/15/95</u>
	Print	Signature	Date
Technical Reviewer:	<u>Allen W. McLaurin</u>	<u>Allen W. McLaurin</u>	<u>12-18-95</u>
	Print	Signature	Date
PI Approval:	<u>Hans W. Papenguth</u>	<u>Hans W. Papenguth</u>	<u>12-18-95</u>
	Print	Signature	Date
QA Approval:	<u>SUSAN Y. PICKERING</u>	<u>Susan Y. Pickering</u>	<u>12-18-95</u>
	Print	Signature	Date
Records Center Approval:	<u>S.E. SHARPTON</u>	<u>SE Sharpton</u>	<u>12/15/95</u>
	Print	Signature	Date

1.0 REVISION HISTORY

This document replaces TOP-6119-05 draft 2. The only reason for this revision is to comply with SNLA-WIPP QA requirements.

2.0 PURPOSE

This procedure provides for the calibration, operation, maintenance of the N4MD Sub-Micron Particle Analyzer as part of the laboratory geochemistry research activities in support of the Waste Isolation Pilot Plant (WIPP) Project.

3.0 SCOPE

This procedure is applicable only for the N4MD.

This document is not meant to substitute for the manufacturer's reference manual for the N4MD. The user is responsible for reading and understanding the manual (see references).

4.0 SAFETY

This document does not address ES&H issues. Laboratory ES&H procedures described in the SOPs of the laboratory in which the equipment is used shall be adhered to.

5.0 RESPONSIBILITIES

The Principal Investigator (PI), or designee, whose activities warrant the use of this procedure is responsible for implementing the requirements of this procedure.

The Project Scientist (PS), or designee, is responsible for performing the calibrations and measurements following the requirements of this procedure, documenting calibrations, and assuring that the latest revision of this document is followed.

6.0 CONTROLS

Controls are established by written procedures or instructions prepared in accordance with QAP 5.3, PREPARING, REVIEWING, AND APPROVING TECHNICAL OPERATING PROCEDURES (Revision 1, effective date: 7/31/95) of the Sandia National Laboratories WIPP Quality Assurance Program. Procedures are issued in accordance with QAP 6.1, DOCUMENT CONTROL SYSTEM (Revision 1, effective date: 7/31/95) of the Sandia National Laboratories WIPP Quality Assurance Program.

6.1 STANDARDS

Calibration will be verified using commercially obtained size standards as QC samples.

At this time, no nationally recognized standards (such as from the NIST) are known to exist. The value of the commercially obtained standards will be verified by comparison to the measured value of a standard of independent origin.

The manufacturers, lot numbers and expiration dates (if any) of the standards used shall be recorded in the laboratory notebook.

The standards will not be used past the expiration date listed on the container by the manufacturer.

Standards should be ultrasonicated for about 30 seconds at about 125 watts prior to analysis.

7.0 QUALITY CONTROL

If results of a QC Sample are not within the control boundaries (see section 7.2), all samples bracketed by this QC sample shall be flagged on the data reports and corrective action documented with the data.

7.1 CALIBRATION

There are no means for the user to calibrate the N4MD. The unit was factory-calibrated and its calibration was verified by the installing technician.

Quality Control is implemented through use of performance tests.

7.2 PERFORMANCE TEST CRITERIA

Performance tests will be done by measuring the electrophoretic mobility of a QC sample. A difference between the value of the measurement and the manufacturer's stated value of the QC sample of greater than 10% shall constitute a failed performance test.

7.3 CORRECTIVE ACTION

If a performance test is failed, the cuvet and sample shall be replaced and the test repeated. If the instrument again fails the performance test, the Fault Isolation Table of section 5 the product reference manual (see Appendix 2) shall be consulted and corrective action taken as appropriate. If the instrument still fails its performance test, it shall be tagged and taken out of service until repaired.

Failures of performance tests and the remedial action taken shall be documented on the analysis printout. Failures of more than one performance test in a given day shall be documented in the appropriate scientific notebook.

7.4 FREQUENCY

The instrument's calibration shall be verified with performance tests immediately prior to and immediately after each day's use. If a batch of analyses are done, a performance test will be done at least once every 10 analyses.

8.0 PROCEDURE

Analyses shall be performed as per instructions in section 3.4 of the reference manual (see Appendix 1).

8.1 OPTIMIZATION

When analyzing particles in a diluent in which particles have never before been analyzed with the N4MD, a standard should be analyzed in the new diluent to ensure that the correct viscosity and refractive index values have been entered.

Avoid re-using disposable cuvetts. Avoid touching the lower half of the cuvetts.

The sample concentration should be such that the sample intensity (located under "sample preparation" in the "Input Data" menu) is between 5.00 E+4 and 1.00 E+6 counts per second.

Allow the instrument at least 30 minutes to warm up prior to analysis.

The sample temperature measured by the instrument should be within 2°C of the set temperature

Samples shall be analyzed for at least 30 seconds.

If SAMPLE WARNING is printed in place of the results on the printout, convection currents or bacterial movement may be causing the instrument to give inaccurate results. Check the sample for contamination (especially bacterial contamination) and for temperature equilibration.

If the possibility of particles of varying sizes (*heterodisperse populations*) is a concern, print out the results and check the graph for multiple or broad peaks.

9.0 MAINTENANCE

Maintenance will be performed on the instrument as instructed in the section 5 of the reference manual (see Appendix 2).

10.0 QA RECORDS

Performance test and data printouts will be submitted to the SWCF or the results will be recorded in the laboratory notebook in accordance with Sandia National Laboratories WIPP Quality Assurance Procedure 20-2, "PREPARING, REVIEWING, AND APPROVING SCIENTIFIC NOTEBOOKS" (Revision 1, effective date: 7/31/95).

11.0 REFERENCES

Coulter Electronics, Incorporated, 1985, 1986. *Coulter Model N4MD Product Reference Manual*, Coulter Electronics, Incorporated, Hialeah, FL.

QAP 5.3, PREPARING, REVIEWING, AND APPROVING TECHNICAL OPERATING PROCEDURES (Revision 1, effective date: 7/31/95)

QAP 6.1, DOCUMENT CONTROL SYSTEM (Revision 1, effective date: 7/31/95)

QAP 20.2, PREPARING, REVIEWING, AND APPROVING SCIENTIFIC NOTEBOOKS (Revision 1, effective date 7/31/95)

APPENDIXES

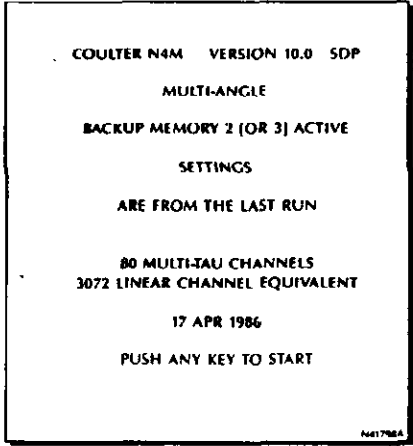
Product Reference Manual - Coulter Model N4MD

APPENDIX 1:	Analysis	26 pages
APPENDIX 2:	Maintenance	4 pages

3.4 STARTUP PROCEDURES (Cont'd)

Prepare for Analysis

The column on the left (ACTION) lists the operator action, and the column on the right explains that action (EXPLANATION). Perform all procedures in the order they are presented.

ACTION	EXPLANATION
<p>1. Move the on/off toggle switch to the right to power up the instrument. Allow the instrument to warm up at least 30 min.</p> <div data-bbox="509 661 919 1108">  </div> <p>Figure 3.4 Status Screen</p> <p>IMPORTANT Careful sample preparation is a key element in obtaining reliable, consistent results. Refer to heading 3.3, Sample Preparation.</p>	<p>1. The instrument's power is on. The status screen, Figure 3.4, appears on the CRT screen. However, if the instrument's lithium battery located on the 3048 board has discharged, the information contained in the battery-backed memory is lost. After entering the date and time, if the battery-backed memory is lost the default values for the instrument display by pressing <SELECT ANALYSIS> and <INPUT DATA>. The expected lifetime of this battery is five years.</p>
<p>2. After the sample is prepared and placed in a clean cuvet (sample must fill the cuvet at least 1/2 full), put the lid on the cuvet. Place the sample cuvet in the sample compartment and push all the way to the bottom.</p>	<p>2. Slide the sample compartment door toward the rear of the unit. An interlocking shutter prevents the laser beam from entering the sample compartment when the lid is open. Place the cuvet in the opening in the bottom of the sample compartment. Close the sample compartment door by sliding it forward.</p>
<p>3. Press any key to display the INPUT DATA menu.</p>	<p>3. Control parameter/selection settings and their current values/selections display on the INPUT DATA menu, Figure 3.5.</p>

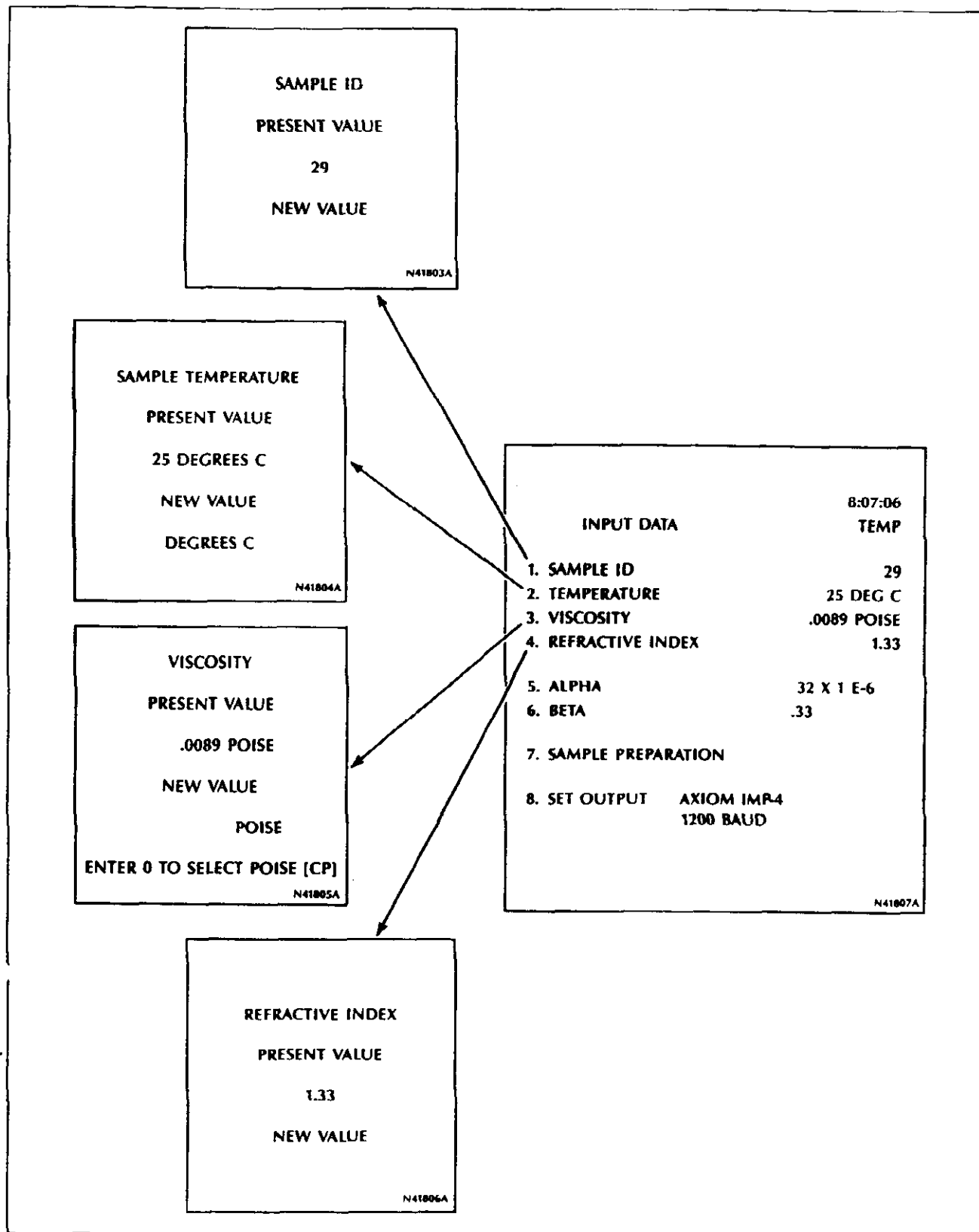


Figure 3.5 Sample Parameter Selection

3.4 STARTUP PROCEDURES

Prepare for Analysis

ACTION	EXPLANATION
3. (Cont'd)	<p>3. Access to adjust any of the settings is accomplished via the screen cursor (>). Advance the cursor until it is adjacent to the setting to be adjusted. The cursor can be advanced in one of two ways:</p> <p>a. Pressing <CLEAR> advances the cursor one position to the next control setting.</p> <p>b. You can advance the cursor directly to a specific control setting by pressing the desired number on the numeric keypad (shown on the display screen).</p>
4. Move the cursor until it is adjacent to SAMPLE ID and press <ENTER>. Enter a set of numbers, from 1 to 17 digits, or decimal points by pressing the selected numeric keys or <.> and <ENTER>.	<p>4. The display screen (SAMPLE ID) appears as shown in Figure 3.5. The sample ID number is equivalent to a run number; it lets you identify a specific run. The sample ID also displays on printouts. By entering a value (a sample descriptive number), a number of periods, and a number (this number increments by one with each run of this sample), each run has its own sample ID number.</p>
5. Press <ENTER> to return to the INPUT DATA menu.	<p>5. The cursor is adjacent to TEMPERATURE on the INPUT DATA menu.</p>

3.4 STARTUP PROCEDURES

Prepare for Analysis (Cont'd)

ACTION	EXPLANATION
<p>6. Press <ENTER> again to access the SAMPLE TEMPERATURE screen.</p> <p>To enter the required temperature, press the numeric keys and decimal point, if applicable, and <ENTER> to implement the new value.</p> <p>Example: To enter 22.4°C, press the following keys in sequence: <2>, <2>, <.>, <4>, and <ENTER>.</p>	<p>6. The SAMPLE TEMPERATURE screen appears as in Figure 3.5. You can select the temperature for sample analysis from 4 to 90°C. The temperature is regulated by an electronic Peltier device. Precision of the temperature stabilization is 0.2°C with the ambient temperature at 25°C. The instrument accepts up to three digits and a decimal point.</p> <p>The new value displays on the SAMPLE TEMPERATURE screen.</p> <p>When the unit is first powered up or when the temperature value is changed, a flashing TEMP message appears in the upper right-hand corner of the screen. This indicator has three modes:</p> <p>Flashing - The sample compartment is not close to the temperature set point.</p> <p>Steady - The sample compartment is close to the set point.</p> <p>Off - The sample compartment has settled at the set point.</p> <p>When introducing a new sample, allow at least 15 min for the temperature to stabilize.</p>
<p>7. Press <ENTER> to return to the INPUT DATA menu.</p>	<p>7. The INPUT DATA menu appears with the cursor adjacent to VISCOSITY.</p>

3.4 STARTUP PROCEDURES

Prepare for Analysis (Cont'd)

ACTION	EXPLANATION
<p>8. With the cursor adjacent to VISCOSITY, press <ENTER>.</p> <p>Enter the diluent viscosity value by first pressing the selected numeric key values and then pressing <ENTER> to implement.</p> <p>Example: If the diluent used is chloroform and the sample temperature is 20°C the diluent viscosity value (see Appendix B) is 0.0058 POISE. Enter by pressing:</p> <p><.), <0>, <0>, <5>, <8>, and <ENTER>.</p>	<p>8. The VISCOSITY screen, Figure 3.5, appears.</p> <p>This parameter is the viscosity of the diluent at the temperature specified in the previous step.</p> <p>The standard unit of this parameter is POISE. The Model N4MD accepts units of centipoise. Press <0> to switch units. The instrument accepts up to four digits and a decimal point.</p> <p>The new value displays on the VISCOSITY screen.</p> <p>Be sure to verify that the appropriate value for the unit is entered.</p>
<p>9. Press <ENTER> to return to the INPUT DATA menu.</p>	<p>9. The cursor is adjacent to REFRACTIVE INDEX.</p>
<p>10. Press <ENTER> again to access the REFRACTIVE INDEX screen.</p> <p>Enter the diluent refractive index by first pressing the numeric keys and then pressing <ENTER> to implement.</p> <p>Example: If the diluent used is chloroform, the diluent refractive index value is 1.446 (see Appendix B). Enter by pressing:</p> <p><1>, <.), <4>, <4>, <6>, and <ENTER>.</p>	<p>10. The REFRACTIVE INDEX screen, Figure 3.5, appears. This parameter is the refractive index of the diluent. The instrument accepts up to four digits and a decimal point, if applicable.</p> <p>The new value displays on the REFRACTIVE INDEX screen.</p>
<p>11. Press <ENTER> to return to the INPUT DATA menu.</p>	<p>11. The cursor is adjacent to ALPHA.</p>

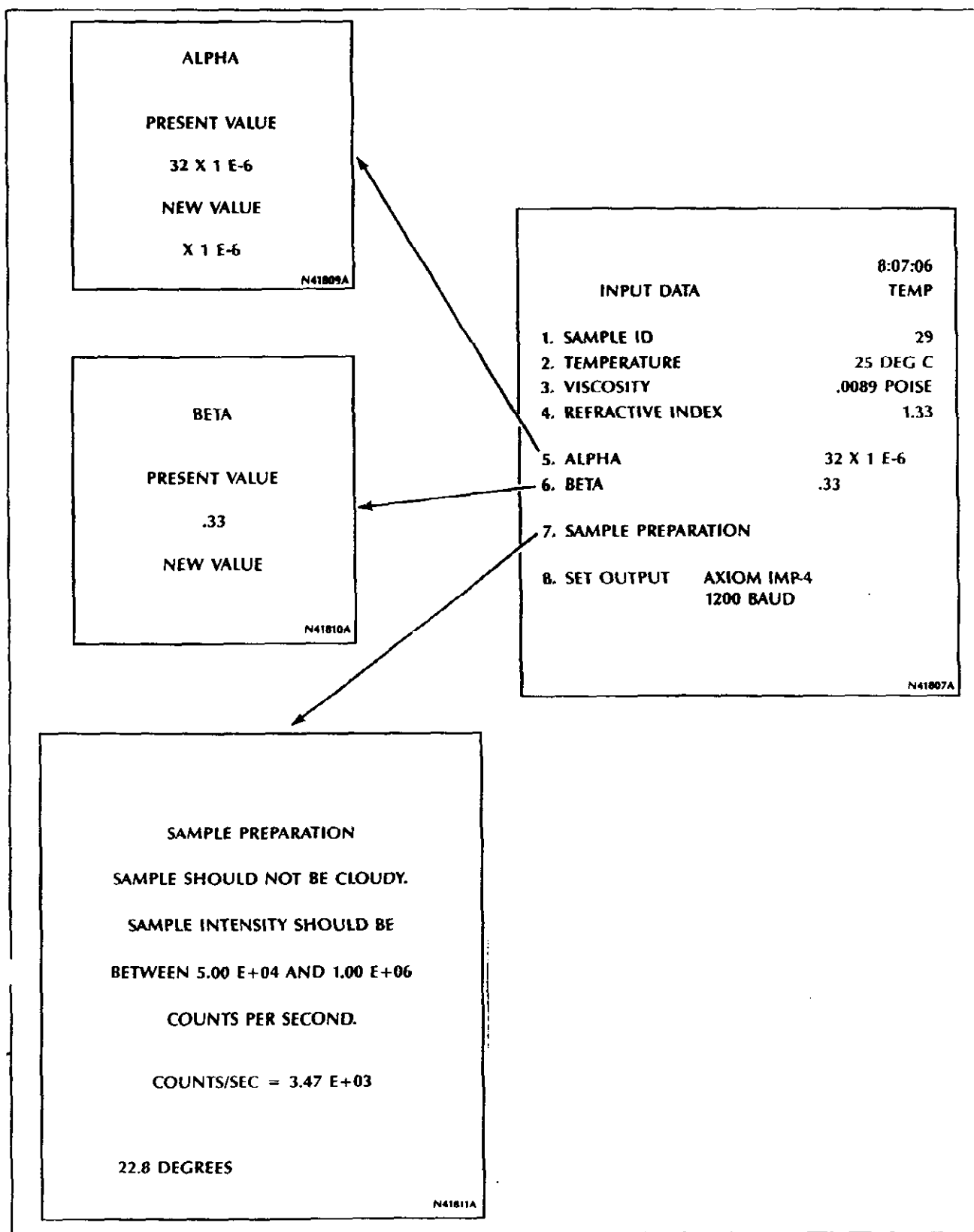


Figure 3.6 Additional Sample Parameter Selections

3.4 STARTUP PROCEDURES

Prepare for Analysis (Cont'd)

ACTION	EXPLANATION
<p>12. Press <ENTER> to access the ALPHA screen.</p> <p>Enter the sample alpha (α) value by pressing the selected numeric keys and <ENTER>.</p> <p>Example: If the sample and diluent are poly(vinyl pyrrolidone) and water, the α value is 1.0×10^{-4} (Appendix B). Enter by pressing:</p> <p><1>, <0>, <0>, and <ENTER>.</p>	<p>12. The ALPHA screen, Figure 3.6, appears. The alpha value is sample dependent and only used in the calculation of molecular weight.</p> <p>The Model N4MD exponent is a fixed term 10^{-6}, so the value 1.0×10^{-4} must be adjusted to read 100×10^{-6}. The instrument accepts up to five digits for an α value.</p> <p>See Appendix B for alpha (α) values for selected polymers, and heading 3.6, Calculation of Alpha and Beta, to determine an α value. If particle diameter is being selected, no value (or change) is needed for alpha or beta; the Model N4MD disregards any values present.</p> <p>The new α value displays on the ALPHA screen.</p>
<p>13. Press <ENTER> to return to the INPUT DATA menu.</p>	<p>13. The cursor is adjacent to BETA.</p>
<p>14. Press <ENTER> again to access the BETA screen.</p> <p>Enter the sample beta value by pressing the selected numeric keys and a decimal point then press <ENTER> to implement.</p> <p>Example: If the sample and diluent are poly(vinyl pyrrolidone) and water, the β value is 0.50 (Appendix B). Enter by pressing:</p> <p><0>, <.>, <5>, <0>, and <ENTER>.</p>	<p>14. The BETA screen, Figure 3.6, appears. The beta (β) value is sample dependent (like the alpha value). See Appendix B for β values for selected polymers and heading 3.6, Calculation of Alpha and Beta, to determine a β value.</p> <p>The instrument accepts three digits plus the decimal point.</p> <p>The new β value displays on the BETA screen.</p>
<p>15. Press <ENTER> to return to the INPUT DATA menu.</p>	<p>15. The cursor is adjacent to SAMPLE PREPARATION.</p>

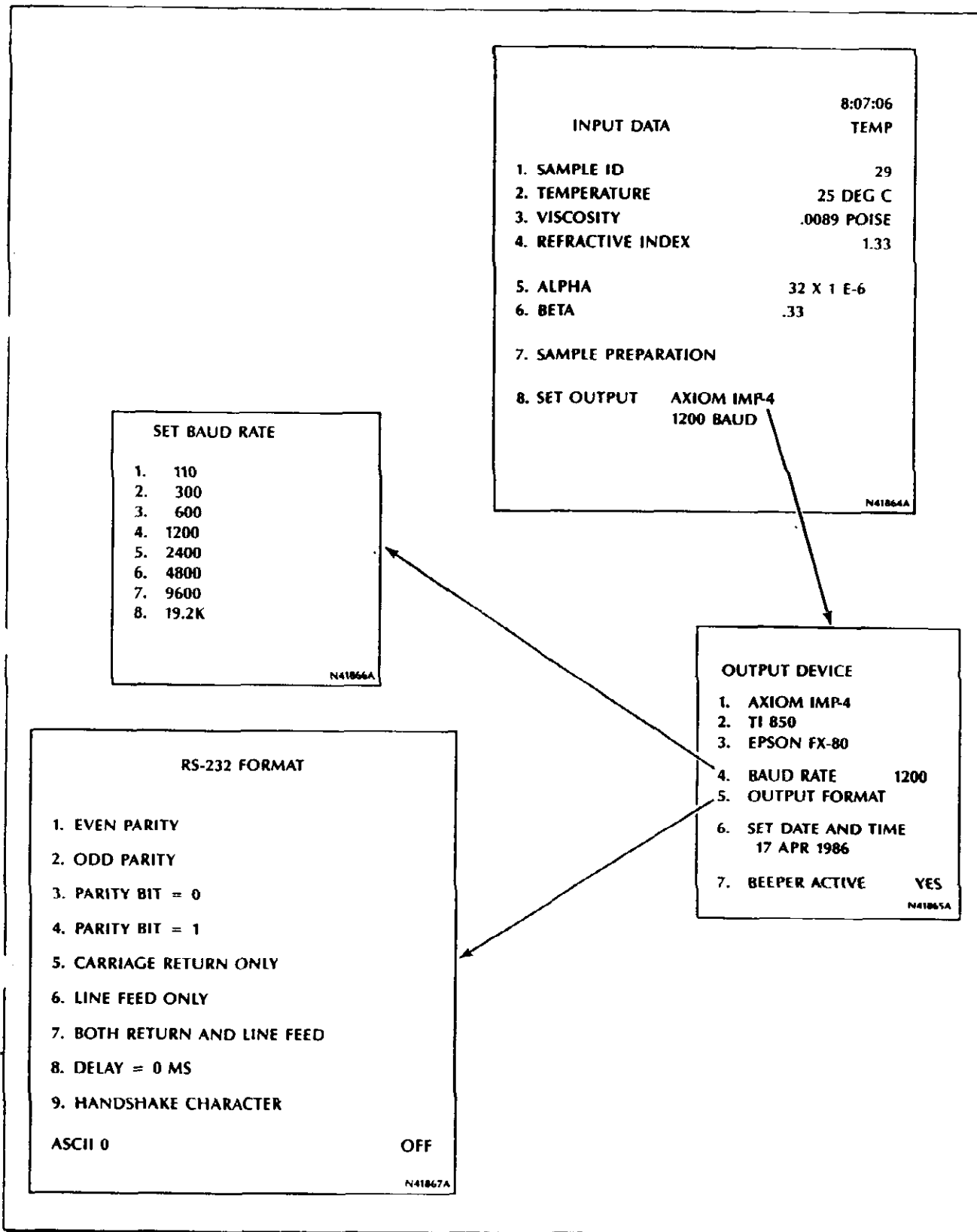


Figure 3.7 Setting the Output

3.4 STARTUP PROCEDURES

Prepare for Analysis (Cont'd)

ACTION	EXPLANATION
16. Press <ENTER> again to access the SAMPLE PREPARATION screen.	<p>16. The SAMPLE PREPARATION screen, Figure 3.6, appears.</p> <p>The sample concentration is measured at the angle of interest and displays this value. If the angle displayed is not the desired angle, press <ANGLE> and reselect an angle. Then access the SAMPLE PREPARATION screen to check the concentration.</p> <p>The sample should not be cloudy. If the concentration is high, the intensity counts may be low (due to the absorption of scattered light by other particles). Read the bottom line of the screen and verify that the sample intensity (counts/s) is between $5.00 \text{ E}+04$ and $1.00 \text{ E}+06$ counts/s. If the sample intensity is not within this range, increase or decrease the concentration as appropriate and repeat.</p>
17. Press <ENTER> to return to the INPUT DATA menu.	17. The cursor is adjacent to SET OUTPUT. When using a printer or data terminal you must select an output device.
18. Press <ENTER> again to access the OUTPUT DEVICE menu.	<p>18. The OUTPUT DEVICE menu, Figure 3.7, appears.</p> <p>The Model N4MD software automatically sets the output format for the Axiom IMP-4, the II-850, and the Epson FX-80 printers when selected. To interface to an Axiom IMP-4, the cursor should be adjacent to AXIOM IMP-4; then press <ENTER>. The II-850 and Epson FX-80 printers are interfaced the same way. No other output setting is required for these three printers. See Appendix C for printer switch settings and cable assemblies. The Model N4MD has (as a standard feature) an RS-232C interface.</p>

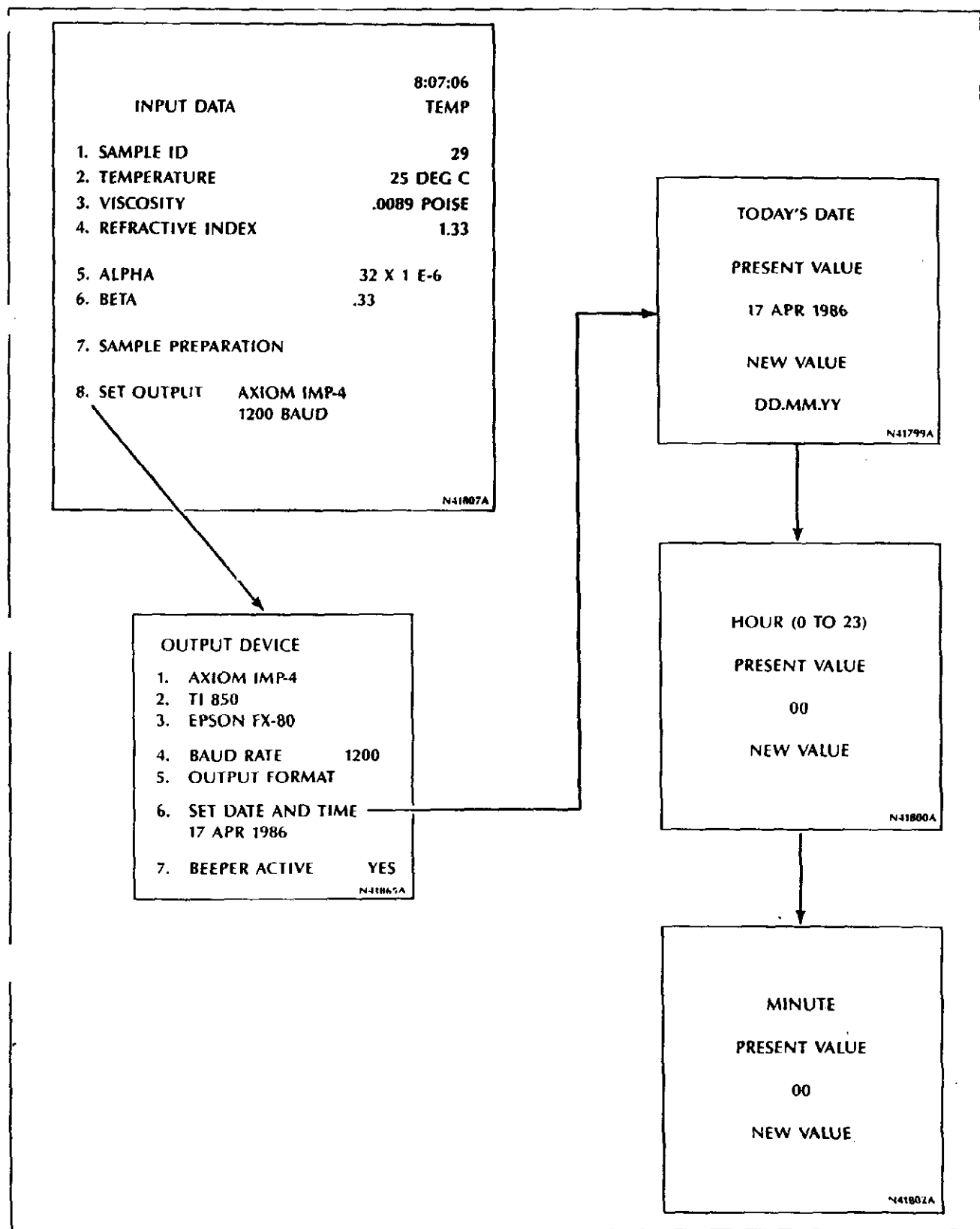


Figure 3.8 Inputting the Date and Time

3.4 STARTUP PROCEDURES

Prepare for Analysis (Cont'd)

ACTION	EXPLANATION
<p>19. Move the screen cursor adjacent to BAUD RATE and press <ENTER> to select the RS-232C interface.</p> <p>Move the cursor to the required setting and press <ENTER> to set the RS-232C baud rate.</p>	<p>19. The SET BAUD RATE menu, Figure 3.7, appears.</p> <p>The baud rate that you enter depends upon the specific external device (printer or computer) to which the Model N4MD is interfaced. Once the data transmission rate is entered for that device it is normally not reset. Consult the external device instruction manual for necessary data transfer rate.</p>
<p>20. Move the cursor to OUTPUT FORMAT, Figure 3.7, and press <ENTER>.</p>	<p>20. The RS-232 FORMAT menu, Figure 3.7, appears. Each of the RS-232C settings is user selectable, depending on particular needs. Selections 1 through 4 offer you a full range of parity settings. Selections 5 through 7 define three specific character combinations that are used to simplify data communication with external devices requiring one of these settings. Selection 8 lets you select a time delay (0 to 9999 ms) between transmission of data lines. Selection 9 is used in 'handshaking systems'. Allowable values are 0 to 127. Setting the ASCII characters to 0 indicates no handshaking is to occur.</p> <p>All INPUT DATA values are entered.</p>
<p>21. Move the cursor to SET DATE AND TIME, Figure 3.7, and press <ENTER>.</p>	<p>21. The TODAY'S DATE and subsequent screens, Figure 3.8, appear.</p> <p>This lets you reset these parameters without the instrument being shut off and restarted.</p>
<p>22. Enter the correct date by pressing the appropriate numeric keys, <.>, and <ENTER>. Refer to Figure 3.8.</p>	<p>22. TODAY'S DATE is displayed. The date is recorded at the beginning of a printout.</p>

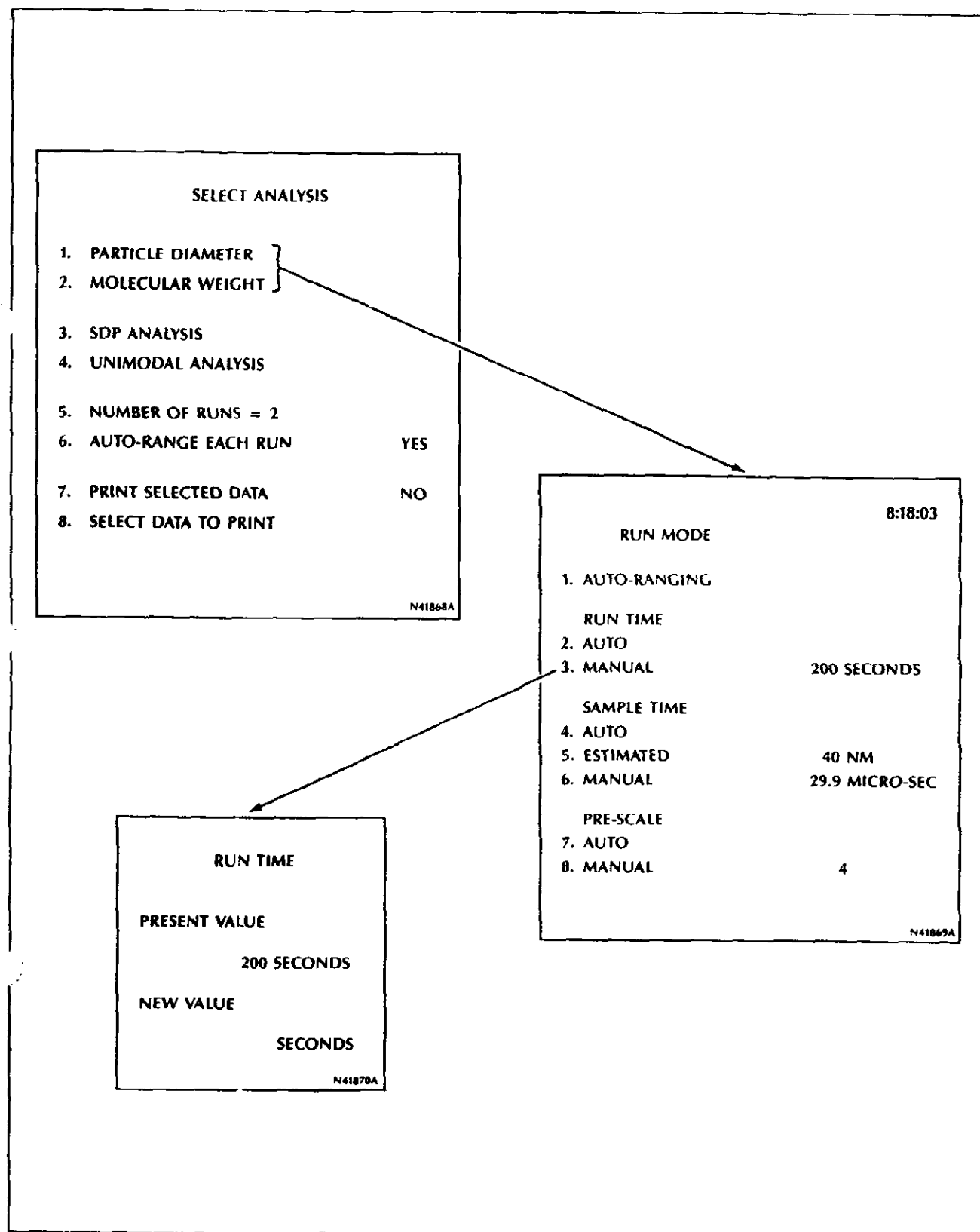


Figure 3.9 Selecting the Run Time

3.4 STARTUP PROCEDURES

Prepare for Analysis (Cont'd)

ACTION	EXPLANATION
23. Press any key to access the HOUR display. To enter the hour of the day (9:45), press the required numeric key <9> and <ENTER>.	23. The HOUR (0 TO 23) display appears. The hour selected (9) is displayed.
24. Press any key to access the MINUTE display. To select the minutes value for the correct time (9:45), press the appropriate numeric keys <4> and <5>, and <ENTER>.	24. The MINUTE display appears. The new value (45) is entered. You are now finished setting the date and time.
25. Move the cursor to BEEPER ACTIVE, Figure 3.7.	25. Toggle your selection via <YES> or <NO>. Each time a key is pressed a beep is emitted. If you choose to remove this feature, change the setting to NO.

Select Analysis

You can select either of two types of measurement:

PARTICLE DIAMETER, or
MOLECULAR WEIGHT.

ACTION	EXPLANATION
1. Press <SELECT ANALYSIS>.	1. The SELECT ANALYSIS menu, Figure 3.9, appears. The cursor is adjacent to PARTICLE DIAMETER.
2. Select measurement of either PARTICLE DIAMETER or MOLECULAR WEIGHT. Move the cursor adjacent to your selection and press <ENTER>.	2. The RUN MODE menu, Figure 3.9, appears. The selections, PARTICLE DIAMETER and MOLECULAR WEIGHT, let you select the sample parameters determined and presented as results on the screen. In addition, you select the form of display.

3.4 STARTUP PROCEDURES

Select Analysis (Cont'd)

ACTION	EXPLANATION
<p>3. Decide the running mode for each parameter.</p>	<p>3. Both the particle diameter and the molecular weight data are determined from the acquisition of the autocorrelation function of the sample. The specific parameters of the sample entered via the INPUT DATA menu are used where appropriate. To acquire the autocorrelation function of a sample, three run parameters must be specified.</p> <p>a. Run Time: the time interval for data collection and the autocorrelation function is calculated.</p> <p>b. Sample Time: the base period of time that counts are accumulated in each channel. The time is in microseconds (μs).</p> <p>c. Prescale: a parameter that adjusts the signal processing of the instrument to its optimum level.</p> <p>The settings of the three run parameters can be performed in any one of three modes:</p> <p>a. Autoranging: for both particle diameter and molecular weight the instrument automatically determines these parameters.</p> <p>b. Estimated: for both particle diameter and molecular weight you enter a value of an estimate of the sample. The instrument interprets the optimum sample time based on the estimate.</p> <p>c. Manual: you must directly enter the three run parameters.</p>

3.4

Select Analysis

a. Automatic mode

3.4 STARTUP PROCEDURES

Select Analysis

ACTION	EXPLANATION
5. Setting the run time; move the cursor adjacent to your selection and press <ENTER>. (Cont'd)	
b. Manual mode	b. The RUN TIME screen, refer to Figure 3.9, appears.
1) Enter the required run time in seconds. The Model N4MD accepts up to four digits. Press <ENTER> to implement.	1) In general, the longer the run time, the more accurate the results. The new run time displays immediately. See Table 3.3 for estimated run times for unimodal analysis.
2) Press <ENTER> again to return to the RUN MODE menu.	2) The RUN MODE menu, Figure 3.10, reappears. The cursor is adjacent to SAMPLE TIME's AUTO.

IMPORTANT

This table is intended for unimodal analysis ONLY. Do NOT use it for SDP analysis.

TABLE 3.3 ESTIMATED RUN TIMES FOR UNIMODAL ANALYSIS

Diameter in Nanometers	*Seconds to Achieve Run-to-Run CV of:				
	1%	2%	3%	4%	5%
100	170	60	60	60	60
200	340	90	60	60	60
300	510	130	60	60	60
400	680	170	80	60	60
500	850	220	100	60	60
600	1020	260	120	70	60
700	1190	300	140	80	60
800	1360	340	160	90	60
900	1530	390	170	100	70
1000	1700	430	190	110	70
1100	1870	470	210	120	80
1200	2040	510	230	130	90
1300	2210	560	250	140	90
1400	2380	600	270	150	100
1500	2550	640	290	160	110
1600	2720	680	310	170	110
1700	2890	730	330	190	120
1800	3060	770	340	200	130
1900	3230	810	360	210	130
2000	3400	850	380	220	140
2100	3570	900	400	230	150
2200	3740	940	420	240	150
2300	3910	980	440	250	160
2400	4080	1020	460	260	170
2500	4250	1070	480	270	170

*Times have been rounded up to the nearest 10 s. Those times below 1 min have been rounded to 60 s.

3.4 STARTUP PROCEDURES

Select Analysis (Cont'd)

ACTION	EXPLANATION
6. Setting the sample time; move the cursor adjacent to your selection and press <ENTER>.	6. For unimodal samples, the sample time is normally set to the Automatic mode for the first few runs, then set to the Estimated mode once the estimated size or weight is determined. For multimodal samples, the Automatic mode is commonly used. Manual setting of the sample time is frequently used by experimental physicists and chemists. Acquiring a good understanding of photon correlation spectroscopy is encouraged for use of the Manual mode for sample time.
a. Automatic mode	a. The sample time is set automatically.
b. Estimated mode	b. The ESTIMATED DIAMETER screen, Figure 3.10, appears. (The MOLECULAR WEIGHT screen appears if running a molecular weight analysis.)
1) Enter the estimated particle diameter in nanometers (or estimated molecular weight in daltons). Press <ENTER> to implement the value.	1) The instrument accepts up to a four-digit estimate (five digits for molecular weight). The new value is implemented and displayed on the ESTIMATED DIAMETER screen.
2) Press <ENTER> to return to the RUN MODE menu.	
c. Manual mode	c. The SAMPLE TIME screen, Figure 3.10, appears. The sample time can be selected manually by directly entering the time in microseconds.
1) Enter the new value for SAMPLE TIME in microseconds. Press <ENTER> to implement.	1) The instrument accepts up to a four-digit figure (the range of values is 0.063 to 4080 μ s set to two digits of accuracy).
2) Press <ENTER> again to return to the RUN MODE menu.	

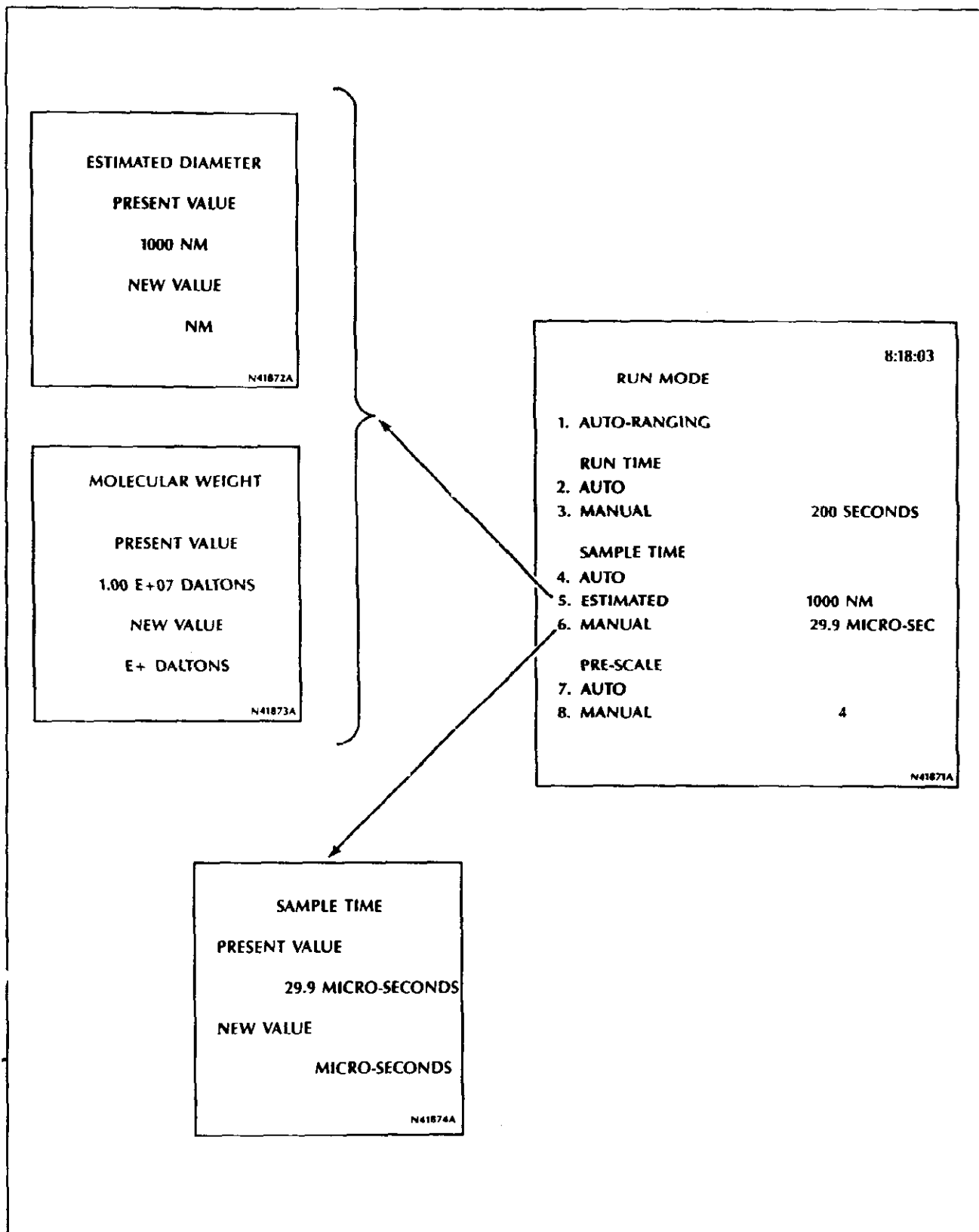


Figure 3.10 Selecting the Sample Time

3.4 STARTUP PROCEDURES

Select Analysis (Cont'd)

ACTION	EXPLANATION
<p>7. Setting the PRE-SCALE's mode; move the cursor adjacent to your selection and press <ENTER>.</p> <p>a. Automatic mode</p> <p>b. Manual mode</p> <p>Select a PRE-SCALE value from the menu by advancing the cursor to the required value and pressing <ENTER>.</p>	<p>7. Prescale should be set to the Automatic mode, and should only be set to the Manual mode for diagnostic reasons. The PRE-SCALE function scales the number of counts down to a value that the hardware can count without overflowing its circuits. If results are not reproducible and the sample is cloudy, look at the RUN MODE menu to see the prescale factor the instrument selected.</p> <p>If a low prescale value was selected for a sample with an obviously high concentration (therefore, a high count rate), the sample may be so overconcentrated that the scattered light is being rescattered before it reaches the photomultiplier. If a prescale of 128 or more is selected, dilute the sample. Ideally, samples should be of a concentration that results in a prescale value of 1 or 2.</p> <p>a. The PRE-SCALE value is automatically set.</p> <p>b. The SET PRE-SCALE menu, Figure 3.11, appears.</p> <p>The PRE-SCALE value is set and displays on the RUN MODE menu.</p>
<p>8. Return to the SELECT ANALYSIS menu by pressing <ENTER>.</p>	

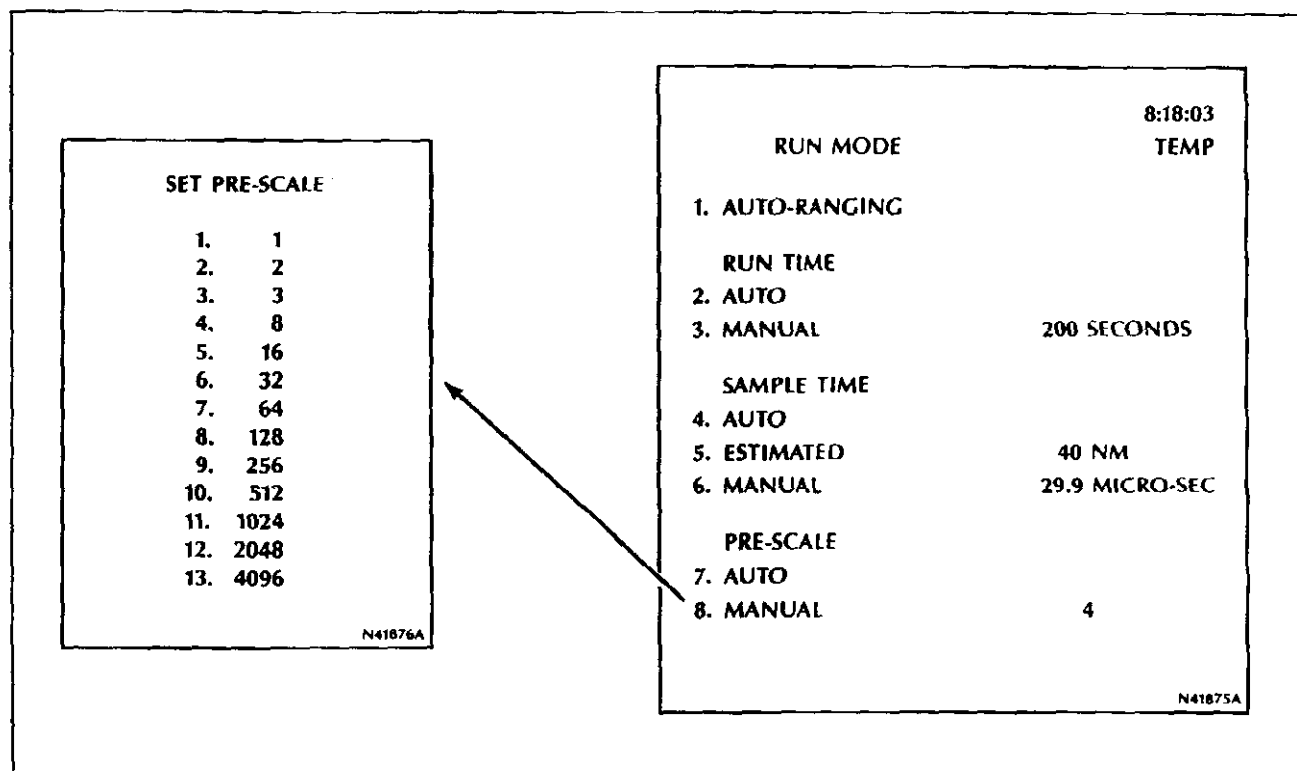


Figure 3.11 Setting the PRE-SCALE Factor

3.4 STARTUP PROCEDURES

Select Analysis (Cont'd)

ACTION	EXPLANATION
<p>9. Press <4> and <ENTER> to select UNIMODAL ANALYSIS.</p> <p>Press <ENTER> again to begin unimodal analysis.</p>	<p>9. PUSH 'ENTER' TO RUN UNIMODAL ANALYSIS displays.</p> <p>If <ENTER> is pressed, unimodal analysis calculations are made on the preexisting correlation data, and the screen display indicates that analysis is in progress.</p> <p>If you want to run unimodal analysis on a new set of correlation data values, proceed to the next step.</p>
<p>10. Press <SELECT ANALYSIS> to return to the SELECT ANALYSIS menu.</p>	<p>10. The NUMBER OF RUNS lets you run the sample more than once, thus increasing the statistical accuracy of results.</p>

3.4 STARTUP PROCEDURES

Select Analysis (Cont'd)

ACTION	EXPLANATION
<p>11. Press <5> and <ENTER> to access the NUMBER OF RUNS screen.</p> <p>Enter the numeric value and press <ENTER> to select a new value.</p>	<p>11. The NUMBER OF RUNS screen, Figure 3.12, appears. The instrument accepts one to three digits (1 to 999).</p>
<p>12. Move the cursor to AUTO-RANGE EACH RUN and press either <YES> or <NO>.</p>	<p>12. You have the option of autoranging for each run of a multirun cycle or autoranging on the first run only. The purpose of this option is to allow sequential measurements of a sample that is changing as a function of time. If autoranging parameters are selected, they are reevaluated at the beginning of each run. It is generally not necessary to autorange for each run if the sample is the same. Only if there is a change in sample (or optional angle) should you autorange each run.</p>
<p>13. Move the cursor to PRINT SELECTED DATA on the SELECT ANALYSIS menu, and press either <YES> or <NO> to select whether the instrument automatically prints out the selected data.</p>	<p>13. YES or NO displays, depending on your choice. If the number of runs is greater than 1, or if the AUTO ANGLE STEP is selected, the Model N4MD is automatically set to YES. You can override this decision by pressing <NO>.</p>
<p>14. Move the cursor to SELECT DATA TO PRINT on the SELECT ANALYSIS menu and press <ENTER> to select the data to be printed automatically.</p>	<p>14. The SELECT DATA TO BE PRINTED menu, Figure 3.12, appears.</p>
<p>15. Move the cursor adjacent to the selection(s) and press <YES> or <NO>.</p>	<p>15. Your selection(s) displays.</p>

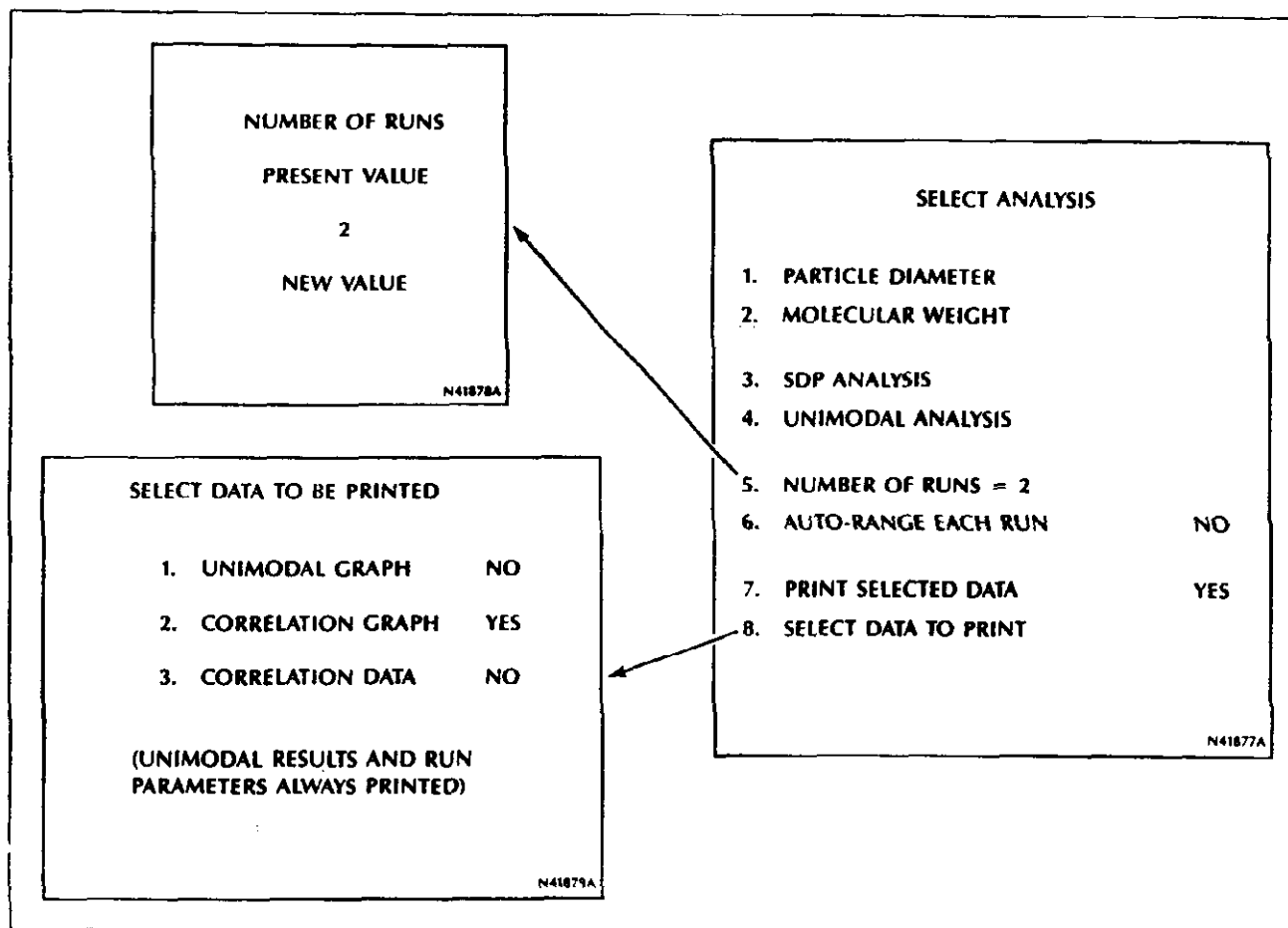


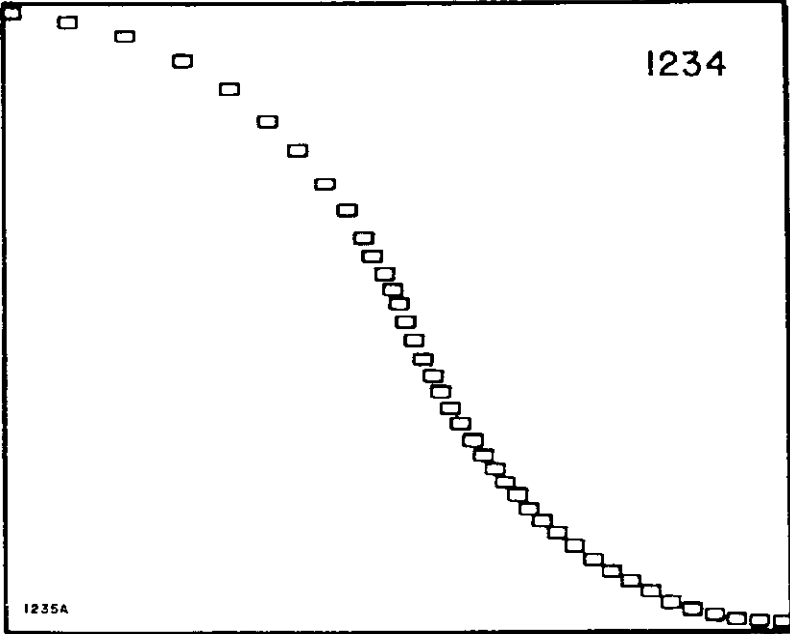
Figure 3.12 SELECT ANALYSIS Menu Selections

3.5 DATA ACQUISITION AND OUTPUT DISPLAYS

Once you have completed the startup procedures, the instrument is ready to perform a data run.

ACTION	EXPLANATION
1. Press <START> to begin sample analysis.	<p>1. If parameters are set to auto-range, the word AUTO-RANGING appears on the screen while the instrument determines the necessary run parameters.</p> <p>In the Manual mode the display appears as shown in Figure 3.13. The display shows the autocorrelation function as it is accumulated. The ordinate is the relative value of the autocorrelation function in each of the 80 channels that represent the multiples of the sample time.</p> <p>In the upper right of the screen, a countdown timer displays the remaining run time in seconds.</p>

3.5 DATA ACQUISITION AND OUTPUT DISPLAYS

ACTION	EXPLANATION
<p>1. Press <START> to begin sample analysis. (Cont'd)</p>  <p>Figure 3.13 Autocorrelation Function Graph</p>	<p>When the instrument has calculated the autocorrelation function for the specified run time, the screen then changes automatically to present the results of the specified analysis.</p> <p>There is a short delay from the time the results display to the time the microprocessor responds to commands from the keyboard.</p> <p>When the run parameters are properly selected and the sample is properly prepared, the autocorrelation curve has an 'S' shape (Figure 3.13). The curve consists of a distinguishable but short plateau in the upper left-hand part of the curve, followed by a descending region, and then a longer plateau on the lower right-hand side. The longer plateau should approach the baseline.</p>
<p>2. Press <CONTINUE>.</p>	<p>2. When making <u>single</u> runs at <u>one</u> angle, this key lets you continue accumulating data instead of resetting the autocorrelation function to zero after a run. You can accumulate data and monitor whether data run time was long enough. No input parameter settings, except run time, can be changed. Displays CONTINUE NOT ALLOWED if any input parameter (except run time) changed.</p> <p>Use this key when first testing an unknown sample. Compare the histogram from the original run time and the one obtained after pressing <CONTINUE>. If the histograms were stable, the correct run time was set. If not, continue to press <CONTINUE> until the histograms stabilize. This is the correct run time for this type of sample.</p>

3.5 DATA ACQUISITION AND OUTPUT DISPLAYS (Cont'd)

ACTION	EXPLANATION
3. Press <SELECT ANALYSIS> to access the screen for selection of either PARTICLE DIAMETER or MOLECULAR WEIGHT.	3. If the analysis selected is unimodal and PARTICLE DIAMETER is selected (SELECT ANALYSIS menu), you can calculate the molecular weight using the results of the sample analysis; likewise for particle diameter, if MOLECULAR WEIGHT is selected.
4. Press <OUTPUT DISPLAYS> to observe the displays.	<p>4. The alternative results display. Table 3.4 shows the organization of the unimodal output displays and the reference figure numbers.</p> <p>The RUN PARAMETERS screen contains a summary of the selections used on this run. There is an A, M, E, or C printed to the right of the screen for some of the values, this indicates whether the value was selected automatically, manually, estimated, or continued. If there is a LOW or HIGH flag on COUNTS/S.T., this indicates COUNTS/S.T. = 0.2 or 5.0, respectively. If SAMPLE WARNING is printed in place of the results on the printout, you need to check the sample for contamination, especially bacterial contamination, and for temperature equilibration. The instrument is not receiving clear Brownian motion signals; they are being confused possibly by convection currents or bacterial movement, something other than Brownian motion.</p>

TABLE 3.4 OUTPUT DISPLAYS TABLE

UNIMODAL OUTPUT DISPLAYS	Ref. Fig. No.
UNIMODAL RESULTS	3.14
PARTICLE DIAMETER UNIMODAL DISTRIBUTION GRAPH	3.16
RUN PARAMETERS	3.15
AUTOCORRELATION CURVE	3.13
AUTOCORRELATION DATA PRINTOUT CONTROL	3.17a
SELECTED DATA PRINTOUT CONTROL	3.17b

UNIMODAL RESULTS		
SAMPLE ID	=	7
MEAN DIAMETER	=	143 NM
95% LIMITS	=	142 TO 144 NM
STANDARD DEVIATION	=	50 NM
MU2/GAMMA SQ	=	.021
DIFFUSION COEFFICIENT	=	3.43 E-08 CM**2/SEC

A. PARTICLE DIAMETER

UNIMODAL RESULTS		
SAMPLE ID	=	7
MEAN MOL. WT.	=	1.49 E09 DALTONS
95% LIMITS	=	1.48 E09 TO 1.49 E09 DALTONS
STANDARD DEVIATION	=	1.44 E09 DALTONS
MU2/GAMMA SQ	=	.021
DIFFUSION COEFFICIENT	=	3.43 E-08 CM**2/SEC

B. MOLECULAR WEIGHT

Figure 3.14 Unimodal Results

RUN PARAMETERS		
SAMPLE ID	=	7
TEMPERATURE	=	20 DEG C
VISCOSITY	=	.01 POISE
REFRACTIVE INDEX	=	1.34
ANGLE	=	90.0 DEGREES
ALPHA = 32 E-6	BETA =	.33
SAMPLE TIME	=	27.2 MICROSEC A
PRE-SCALE	=	4 A
RUN TIME	=	30 SECONDS M
COUNTS/S.T.	=	0 LOW
COUNTS/SEC	=	2.52 E+05
END OF RUN	=	1:05:43

Figure 3.15 RUN PARAMETERS Screen

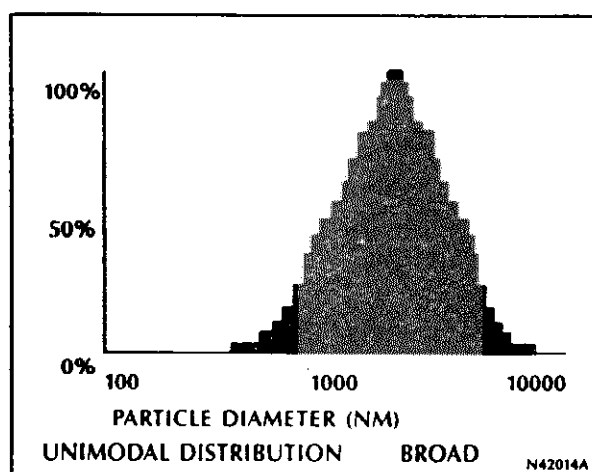


Figure 3.16 Particle Diameter Unimodal Distribution Graph

PUSH PRINT FOR PRINT-OUT OF
CORRELATION DATA VALUES

A. AUTOCORRELATION DATA

PUSH PRINT FOR PRINT-OUT OF
ALL SELECTED DATA SCREENS

B. SELECTED DATA

Figure 3.17 Printout Controls

3.5 DATA ACQUISITION AND OUTPUT DISPLAYS (Cont'd)

Interpreting Autocorrelation Functions

Table 3.5 describes the effects of changing the input parameters. Figure 3.18 illustrates examples of normal and abnormal curves. Table 3.6 references those examples with possible causes.

TABLE 3.5 EFFECTS OF CHANGING PARAMETERS

Parameter Changed	Effects			
	Autocorrelation Curve	Mean	95% Limits	Standard Deviation
REFRACTIVE INDEX DECREASED	none	results decrease	results decrease	results decrease
VISCOSITY DECREASED	none	results increase	results increase	results increase
TEMPERATURE INCREASED	raises curve off baseline	results increase	results increase	results increase
RUN TIME DECREASED	less accurate	less accurate	broadens	results increase
RUN TIME INCREASED	more accurate	more accurate	narrow; more reproducible	results decrease
SAMPLE TIME INCREASED	drops to baseline	erratic results	broadens	results increase
SAMPLE TIME DECREASED	above baseline	erratic results	broadens	results increase
PRE-SCALE DECREASED	disjointed curve	erratic results	broadens	results increase
PRE-SCALE INCREASED	erratic	erratic results	erratic results	erratic results

SERVICE AND MAINTENANCE

The Model N4MD is relatively maintenance free. A clean environment and normal care for the instrument helps protect its working parts.

CAUTION

Do not operate the instrument on a laboratory bench or other surface subject to vibrations.

5.1 FAULT ISOLATION

Table 5.1 lists messages displayed when an error or other inaccuracy is detected, probable causes, and possible solutions.

TABLE 5.1 FAULT ISOLATION

Message	Cause	Solution
TEMP flag during run.	Programmed temperature has not been reached.	Allow enough time for the TEMP flag to be turned off prior to analysis (about 15 min).
HIGH or LOW flag displays on RUN PARAMETERS screen.	1. Concentration of sample is too high or too low.	1. Change sample concentration. Dilute or concentrate sample to bring it into the acceptable scattered intensity range.
	2. Prescale value or sample time value is inappropriate.	2. Lower prescale value if LOW flag appears. Increase prescale value if HIGH flag appears.
SDP ERROR message displays with error code in left-hand corner of screen.	SDP cannot analyze data.	Rerun sample. If error message repeats, run a PSL standard to verify proper instrument functioning. If an error message occurs with the PSL standard, call Coulter Customer Services. If PSL standard is sized correctly, the sample is the problem. Filter the sample, increase the run time, and be sure temperature is equilibrated.

5.1 FAULT ISOLATION

TABLE 5.1 (Cont'd)

Message	Cause	Solution
When <OUTPUT DISPLAYS> is pressed, NO SDP RESULTS displays.	SDP is selected, but the SDP analysis is not yet run.	Run the SDP analysis.
VERY BROAD DISTRIBUTION displays (unimodal analysis).	Distribution is too broad to be analyzed by unimodal program. (MU2/GAMMA SQ>.33)	Verify that the cuvet is clean and the sample is not contaminated. Increase run time or use <CONTINUE>.
Blinking ANGLE ERROR flag.	Multiangle optics misaligned.	Press <ANGLE>. Open sample chamber door. Select RESET ANGLE. Optics automatically realign.
AUTO-RANGING message displays on screen.	Run parameters being determined by the microprocessor.	Allow time for completion.
NARROW message displays on the UNIMODAL RESULTS screen.	Unimodal analysis distribution is too narrow; no SD can be calculated.	Double run time and use <CONTINUE>.
MEMORY FAILURE message displays on the initial power up screen.	Instrument fails the RAM memory test.	Call Coulter Customer Services.
RANGE WARNING message displays on the SDP RESULTS screen.	A large number of particles border the size range selected.	Select a broader size range.
NOT ENOUGH DATA displays on the UNIMODAL RESULTS screen.	Run time is too short to accumulate sufficient data.	Double run time and use <CONTINUE>.
SAMPLE WARNING displays on the RUN PARAMETERS screen.	There are problems in the sample that lead to inaccurate analysis; usually there is particle motion other than Brownian motion (convection currents, sound or mechanical vibrations, or bacterial contamination).	Let the sample remain in the sample compartment for at least 10 min before running analysis. Observe and remove any device that can cause sound or mechanical vibration. Use a microscope to observe the sample for bacterial contamination.

5.1 FAULT ISOLATION (Cont'd)

A number of conditions can lead to undesirable components in the correlation function that are not readily apparent in the display; these can lead to misleading results, particularly when SDP analysis is used.

Table 5.2 lists problematic conditions, explanations, and solutions.

TABLE 5.2 POSSIBLE PROBLEMATIC CONDITIONS

Problematic Condition	Explanation	Solution
Overestimating sizes	Dirty cuvet causes stray light.	Clean cuvet.
	Bubbles in solution. Bubbles may not be visible but appear as particles in analysis. (Cold solvents allow gases to escape when warming; therefore, be careful when using cold solvents.)	Debubble solution.
Large DUST Term in SDP Analysis	Dirty cuvet contributes particles to a sample.	Clean cuvet.
	Dirty sample.	Filter or centrifuge sample.
Distorted Auto-correlation Function	Unstable temperatures. Leads to convection currents that distort the correlation function. This condition is very serious at higher temperatures.	Wait longer before starting measurements. Sample should equilibrate in the Model N4MD for 15 min. Alternately, water bath can be used to preequilibrate the sample.
	Large, flexible, or rod-shaped samples. The rotation or bending of the samples adds terms to the autocorrelation function.	Work at a smaller angle.

5.2 ACCESSORIES

The following are lists of part numbers for accessory items. Contact your Industrial Coulter Technical Specialist.

Accessories	CEI PN	CMS PN
Sample Cuvets (Model N4MD, plastic, 200/box)	7800091	
Model N4MD Manual	4235592	N/A
N4 Printer (II-850XL)	6603290	250-170
Precision Glass Cells	7800040	238-816
AccuComp® System for Model N4 Series		
Complete Apple IIe	6603289	241-891
Apple IIe Software	7800110	
IBM Software	7800111	

Model N4 Series Upgrade Kits

Upgrade Kit	CEI PN	CMS PN
N4S to N4SD Upgrade	6603291	250-171
N4SD to N4MD Upgrade	6603292	199-869
N4128MD to N4MD Upgrade	6603332	N/A
N4128SD to N4SD Upgrade	6603333	N/A
N4128S to N4SD Upgrade	6603347	N/A
N4128M to N4MD Upgrade	6603348	N/A
N432S to N4S Upgrade	6603349	N/A
N4SD (9.2 V) to N4SD (10.0 V) Upgrade	7800158	N/A
N4MD (9.2 V) to N4MD (10.0 V) Upgrade	7800189	N/A

CALIBRATORS (Latex)

Size	Name	CEI PN	CMS PN
0.04 μ m	Polystyrene - Carboxy	1615105	156-943
0.09 μ m	Polystyrene	1615106	156-950
0.17 μ m	Polystyrene	1615107	156-968
0.31 μ m	Polystyrene	1615108	156-976
0.5 μ m	Polystyrene	1607008	359-448
0.8 μ m	Polystyrene	1607009	359-455
1.099 μ m	Polystyrene	1607010	359-463
1.304 μ m	Polystyrene	1607011	359-471

Model N4 Series Part Numbers

Description	CEI PN	CMS PN
Model N4S, 115 Vac	6603283	250-172
Model N4S, 230 Vac	6603284	N/A
Model N4SD, 115 Vac	6603285	199-273
Model N4SD, 230 Vac	6603286	N/A
Model N4MD, 115 Vac	6603287	199-315
Model N4MD, 230 Vac	6603288	N/A